Int J Clin Exp Med 2014;7(11):4191-4198
www.ijcem.com /ISSN:1940-5901/IJCEM0002317

*Original Article*

# Serum cytokeratin 18 fragment level as a noninvasive biomarker for non-alcoholic fatty liver disease

Yuta Aida, Hiroshi Abe, Yoichi Tomita, Tomohisa Nagano, Nobuyoshi Seki, Tomonori Sugita, Munenori Itagaki, Haruya Ishiguro, Satoshi Sutoh, Yoshio Aizawa

*Department of Internal Medicine, Division of Gastroenterology and Hepatology, Jikei University School of Medicine, Katsushika Medical Center, 6-41-2 Aoto, Katsushika-ku, Tokyo 125-8506, Japan*

Received September 4, 2014; Accepted October 23, 2014; Epub November 15, 2014; Published November 30, 2014

**Abstract:** Background and Aim: We evaluated the usefulness of serum cytokeratin 18 fragment (CK18-F) as a non-invasive biomarker in differentiating nonalcoholic steatohepatitis (NASH) from nonalcoholic fatty liver (NAFL) since the prognosis of the 2 diseases differ. Methods: 116 Japanese patients with nonalcoholic fatty liver disease (NAFLD) proven by liver biopsy were studied. Histological findings were classified according to the NAFLD activity score (NAS) proposed by the Nonalcoholic Steatohepatitis Clinical Research Network. The correlation between histological findings and serum CK18-F levels was investigated. Results: Serum CK18-F levels showed a positive correlation with histologic steatosis ($\rho = 0.271$, $P = 0.0033$), inflammation ($\rho = 0.353$, $P = 0.0005$), ballooning ($\rho = 0.372$, $P = 0.0001$), and the total NAS ($\rho = 0.474$, $P = 2.68 \times 10\text{-}7$). The serum CK18-F level was significantly lower for NAFL (NAS $\leq 2$) than for borderline NASH (NAS of 3-4) or definite NASH (NAS $\geq 5$) ($P = 0.0294$, $P = 1.163 \times 10\text{-}5$, respectively). The serum CK18-F level was significantly higher for definite NASH than for borderline NASH ($P = 0.0002$). The area under the receiver operating characteristic curve of serum CK18-F to predict the presence of NAFL and definite NASH was 0.762 and 0.757, respectively. The optimal cut-off point of serum CK18-F for NAFL and definite NASH was 230 and 270 U/L, respectively. The sensitivity, specificity, positive predict value, and negative predict value of serum CK18-F for NAFL were 0.89, 0.65, 0.34, and 0.97, and those for definite NASH were 0.64, 0.76, 0.72, and 0.67, respectively. Accuracies of diagnosis for both NAFL and definite NASH were 0.70. Conclusions: Serum CK18-F could be a clinically useful biomarker to discriminate between NAFL and NASH.

**Keywords:** Nonalcoholic fatty liver disease (NAFLD), serum cytokeratin 18 fragment (CK18-F), nonalcoholic fatty liver disease activity score (NAS), nonalcoholic fatty liver (NAFL), definite nonalcoholic steatohepatitis (NASH)

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and its prevalence is increasing worldwide [1, 2]. Nonalcoholic steatohepatitis (NASH), a progressive form of NAFLD, can develop into cirrhosis, hepatic failure, and hepatocellular carcinoma [3-5]. Approximately 20% of patients with NASH will develop cryptogenic cirrhosis and even end-stage liver disease [6]. In contrast, nonalcoholic fatty liver (NAFL) without inflammation is a non-progressive disease with well prognosis. Therefore, it is important to distinguish NAFL from NASH [6, 7] clinically. Liver histology is the current gold standard for the differential diagnosis and the definition of activity/fibrosis of NASH. However, many patients may not consent to a liver biopsy due to its inherent risks and for ethical reasons.

Instead of a liver biopsy, the grade of steatosis or fibrosis can be estimated using ultrasonography with a specific apparatus, magnetic resonance imaging (MRI), or a Fibroscan. However, use of these non-invasive tools is limited to specialized research centers due to their operating costs [8-11]. Although a combination of routine blood tests or specific fibrosis markers may be useful for estimating the degree of liver fibrosis caused by NASH, NASH activity is not easily determined by non-invasive methods [12-18].

The cytokeratin 18 fragment (CK18-F) which is reacted to M-30 monoclonal antibody is a sero-

**Table 1.** Clinical features of 116 nonalcoholic fatty liver disease patients

| | |
|---|---|
| Demographic data | |
| Number of patients | 116 |
| Sex (Male/Female) | 41/75 |
| Age (years) | 61 (27-82) |
| Body mass index (kg/m$^2$) | 27.2 (18.8-45.9) |
| Laboratory data | |
| White blood cells (/μL) | 5850 (3000-10700) |
| Hemoglobin (g/dL) | 13.8 (10.5-17.7) |
| Pletelets (×10$^4$/μL) | 20.2 (5.0-35.1) |
| Prothorombin time (%) | 92 (55-100) |
| Aspartate aminotransferase (U/L) | 42 (13-256) |
| Alanine aminotransferase (U/L) | 52 (31-266) |
| Cholinesterase (U/L) | 375 (169-559) |
| Alkaline phosphatase (U/L) | 261 (89-573) |
| gamma-glutamyl-transpeptitase (U/L) | 59 (9-263) |
| Albumin (g/dL) | 4.2 (2.5-5.1) |
| Fasting total cholesterol (mg/dL) | 198 (101-343) |
| Fasting low density lipoprotein-cholesterol (mg/dL) | 117 (49-234) |
| Fasting triglyceride (mg/dL) | 139 (40-389) |
| Fasting plasma glucose (mg/dL) | 107 (68-311) |
| Homeostasis model assessment-Insulin Resistance | 2.89 (0.90-23.3) |
| Ferritin (ng/mL) | 167 (7-3641) |
| Alpha-fetoprotein (ng/mL) | 3 (1-18) |

Data expressed as number of patients or median (range).

logical marker of apoptosis and has been associated with severity of liver disease in patients with chronic hepatitis C (CHC) and NAFLD [19-21]. However, the ability for CK18-F to predict disease severity may differ between Japanese and Caucasian populations. Japanese populations have an elevated risk for co-morbid conditions at a lower body mass index (BMI) than Caucasian populations [22], which may be due to ethnic-specific differences in body composition profiles [23] and may influence the effectiveness of CK18-F use. Further study is needed to reevaluate of the significance of CK18-F in relation to histologic findings in Japanese NAFLD patients since the previous report was relatively small scale [21].

The aim of this study was to determine whether serum CK18-F levels correlated with the NAFLD activity score (NAS) [24] and/or fibrosis in Japanese NAFLD patients. In addition, the cut-off value of CK18-F was examined for discriminating between NAFL and NASH.

## Materials and methods

### *Patients*

A total of 116 NAFLD patients admitted to the Jikei University Katsushika Medical Center (Tokyo, Japan) between January 2010 and December 2013 for liver biopsies were enrolled. NAFLD was diagnosed using the following criteria: (1) abnormal alanine aminotransferase (ALT) levels (> 30 U/L) persisting for more than 6 months; (2) no consumption of alcohol or hepatotoxic drugs; (3) the presence of hepatic steatosis on ultrasonography [25] or cirrhosis without steatosis on a liver biopsy where steatosis was indicated in the past; (4) negative results for hepatitis B virus surface antigen, high titer of hepatitis B virus core antibody, or anti-hepatitis C virus antibody; and (5) absence of abnormal serum ceruloplasmin levels and transferrin saturation ratios. NAFLD diagnosis was confirmed by liver biopsy. Body height and weight were measured at admission and BMI was calculated.

This study was approved by the ethics committee of the Jikei University School of Medicine and the Jikei University Katsushika Medical Center and adheres to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

### *Laboratory examination*

Fasting blood samples were obtained early in the morning on the day of the liver biopsy. Regular physical examinations, complete blood counts, and blood chemistries were carried out using standard methods. The serum low-density lipoprotein-cholesterol (LDL-C) concentrations were calculated using the Friedewald formula [26]. Remaining sera were immediately frozen and kept at -80°C until measurement of CK18-F. The homeostasis model assessment for insulin resistance (HOMA-IR) value was cal-

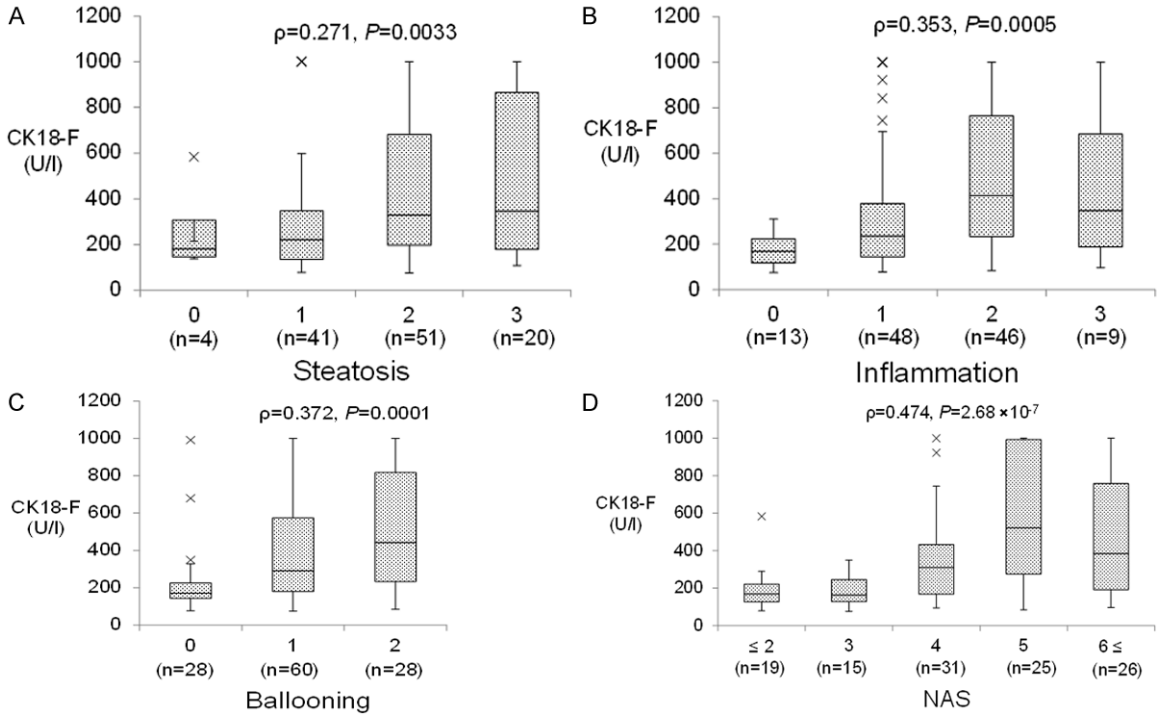


**Figure 1.** Relation between serum cytokeratin 18 fragment (CK18-F) level and nonalcoholic fatty liver disease activity score (NAS).

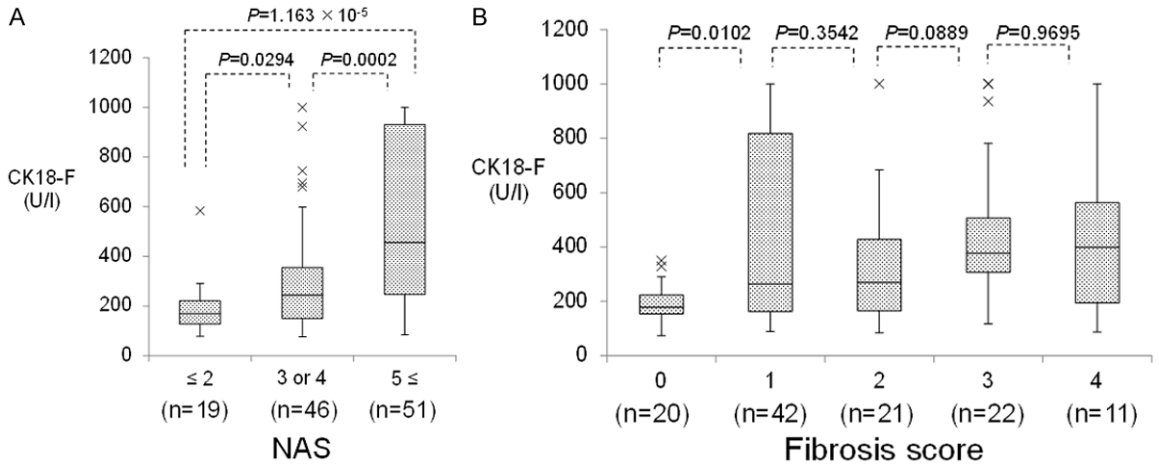


**Figure 2.** Serum cytokeratin 18 fragment (CK18-F) levels according to (A) nonalcoholic fatty liver disease activity score (NAS) and (B) fibrosis score.

culated as fasting glucose (mg/dL) × fasting insulin (μU/mL)/405 [27]. Serum level of CK18-F was measured using the M30-Apoptosense ELISA kit (PEVIVA AB, Bromma, Sweden).

*Histopathologic examination*

Ultrasonography-guided liver biopsies were performed at 2 different sites in the same lobe using a 16-gauge needle. The lengths of the sum of biopsy specimens were more than 1.8 cm. All biopsy specimens were placed in 10% neutral formalin solution for fixation and embedded in paraffin blocks, and sections were cut at 4 μm thickness stained by the hematoxylin-eosin and Masson trichrome. The median number of portal tracts found in each sample was 10 (range 7-12). Histologic findings

were assessed in a blinded fashion by an independent experienced pathologist and were scored according to the staging/grading system proposed by Brunt et al. [24]. Staging of liver fibrosis was scored as follows: stage 0: no fibrosis; stage 1: perisinusoidal or periportal fibrosis with 3 different patterns: 1A: mild, zone 3, perisinusoidal; 1B: moderate, zone 3, perisinusoidal fibrosis; 1C portal/periportal fibrosis; stage 2: perisinusoidal and portal/periportal fibrosis; stage 3: bridging fibrosis; stage 4: cirrhosis. The grade of steatosis was scored from 0 to 3: 0: no steatosis or < 5%, 1: 5-33%, 2: 33-66%, 3: 66% < . Lobular inflammation was classified into 0 to 3: 0: no foci, 1: < 2 foci per 200 × field, 2: 2-4 foci per 200 × field, 3: 4 < foci per 200 × field. Ballooning was graded from 0 to 2: 0: none to rare, 1: few, 2: many. NAS was calculated as an un-weighted sum of the scores for steatosis (0 to 3), lobular inflammation (0 to 3) and ballooning (0 to 2), and ranged from 0 to 8. Cases with NAS of ≤ 2 were diagnosed as NAFL, while cases with NAS of 5 ≤ were diagnosed as definitive NASH. Cases with NAS of 3 and 4 were considered as borderline NASH [24].

*Statistical analyses*

Results were expressed as a number or as median (range). The Mann-Whitney U-test was used to analyze differences between continuous variables. Fisher's exact tests were used to analyze differences in categorical data. Correlation coefficients were calculated using the Spearman rank correlation analysis. The serum CK18-F receiver operating characteristic (ROC) curves were plotted and the area under the ROC curves (AUROCs) calculated to represent their performance to predict NAFL or definite NASH. Statistical significance was determined by applying a two-tailed test and resulted in a *P*-value < 0.05. All statistical analyses were carried out using STATISTICA for Windows version 6 (StatSoft, Tulsa, OK, USA).

## Results

*Clinical characteristics of patients*

The clinical features of 116 NAFLD patients are shown in **Table 1**. The median age was 61 years and median BMI was 27.2 kg/m$^2$.

*Relationship between histologic findings and CK18-F levels*

The median serum CK18-F levels based on steatosis grades 0, 1, 2, and 3 were 182.0, 223.0, 329.0, and 347.0 U/L, respectively. There was a positive correlation between the steatosis grade and the serum CK18-F level (ρ = 0.271, *P* = 0.0033). The median serum CK18-F levels based on inflammation grades 0, 1, 2, and 3 were 169.0, 235.5, 414.0, and 348.0 U/L, respectively. Serum CK18-F levels also showed positive correlation with inflammation grade (ρ = 0.353, *P* = 0.0005). The median serum CK18-F levels based on ballooning grades 0, 1, and 2 were 172.0, 292.0, and 445.0 U/L, respectively. Positive correlation (ρ = 0.372, *P* = 0.0001) was stronger for ballooning grade than steatosis and inflammation grades. In addition, there was a positive correlation between serum CK18-F levels and NAS (ρ = 0.474, $P = 2.68 \times 10^{-7}$) (**Figure 1**).

*Serum CK18-F levels for differentiating between NAFL and definite NASH*

The median of serum CK18-F with NAS of ≤ 2, NAS of 3 or 4 and NAS of 5 ≤ was 169.0, 244.0 and 456.0 U/L, respectively. Serum CK18-F level of NAFL (NAS ≤ 2) was significantly lower than borderline NASH (NAS 3 or 4) and definite NASH (with NAS of 5 ≤) (*P* = 0.0294, $P = 1.163 \times 10^{-5}$, respectively). In addition, serum CK18-F level of definite NASH was significantly higher than that of borderline NASH (*P* = 0.0002) (**Figure 2A**).

*Relationship between serum CK18-F levels and liver fibrosis*

The median serum CK18-F levels of fibrosis stage 0, 1, 2, 3, and 4 were 179.5, 264.5, 270.0, 377.5, and 400.0 U/L, respectively. The CK18-F level for fibrosis stage 0 was significantly lower than that of fibrosis stage 1 (*P* = 0.0102). However, there were no significant relationships between CK18-F levels when comparing fibrosis stages 1 and 2, 2 and 3, or 3 and 4 (**Figure 2B**).

*Predict value of serum CK18-F for NAFL and definitive NASH*

To assess the utility of serum CK18-F levels as a diagnostic tool in differentiating between

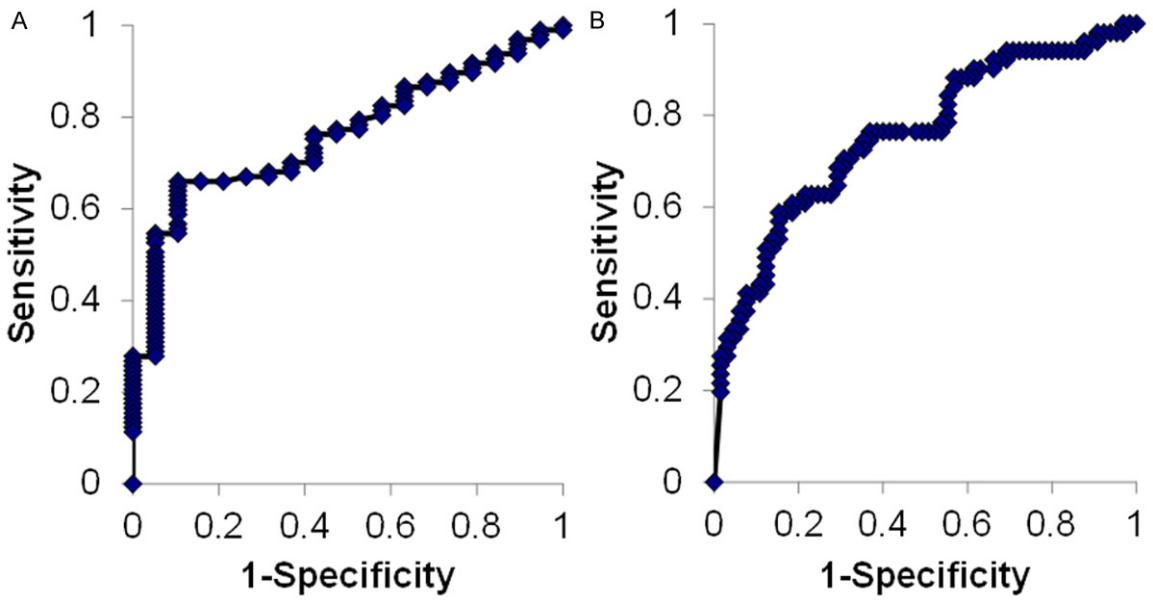


**Figure 3.** A. Receiver operating characteristic (ROC) plot for cytokeratin 18 fragment (CK18-F) in differentiating non-alcoholic fatty liver disease activity score (NAS) ≤ 2 in the total cohort (n = 116). CK18-F had an area under the receiver-operator curve (AUROC) of 0.762 (95% confidence interval [CI]: 0.665-0.859). B. ROC plot for CK18-F in differentiating NAS ≥ 5 in the total cohort. CK18-F had an AUROC of 0.757. (95% CI: 0.667-0.846).

**Table 2.** Performance of CK 18-F to predict NAS 5 ≤ and ≤ 2 in those with determinant value

| | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| CK18 fragment | | | | | |
| 270 < (for NAS 5 ≤) | 0.64 | 0.76 | 0.72 | 0.67 | 0.70 |
| < 230 (for NAS ≤ 2) | 0.89 | 0.65 | 0.34 | 0.97 | 0.70 |

CK 18-F, cytokeratin 18 fragment; NAS, non-alcoholic fatty liver disease activity score NPV, negative predictive value; PPV, positive value.

NAFL and definite NASH, we estimated the AUROC to be 0.762 (95% confidence interval [CI]: 0.665-0.859), and 0.757 (95% CI: 0.667-0.846) (**Figure 3A**, **3B**), respectively. The optimal cut-off values of serum CK18-F for NAFL and definite NASH were 230 and 270 U/L, respectively. The sensitivity, specificity, positive predict value (PPV), and negative predict value (NPV) of the serum CK18-F cut-off value of 230 U/L for NAFL were 0.89, 0.65, 0.34 and 0.97, respectively, and of the serum CK18-F cut-off value of 270 U/L for definite NASH were 0.64, 0.76, 0.72 and 0.67, respectively. Diagnostic accuracies for both NAFL and definite NASH were 0.70 (**Table 2**).

## Discussion

NAFL is a relatively benign form of NAFLD, while NASH commonly causes cryptogenic cirrhosis and may even result in hepatocellular carcinoma [3-5]. The gold standard for the differential diagnosis of NASH or NAFL is liver histology, but many patients do not consent to a liver biopsy.

Serum CK18-F has been reported as a noninvasive biomarker for differentiating NASH from NAFL or predicting the activity of NASH [21, 28-30]. CK18-F is only expressed during apoptosis, while total CK18 could be released from damaged cells during loss of cell membrane integrity [31]. Since CK18-F is generated mainly by caspase 3, which is reportedly activated in a NASH liver, it is conceivable that CK18-F is increased in the sera of NASH patients [32]. It has been reported that serum CK18-F levels were higher in NASH than that in non-NASH patients [21, 28, 29]. However, in the report from Belgium, the value of CK18-F is limited to predict development of NASH. Therefore, the significance of CK18-F on differential diagnosis of NASH has been controversial [33]. There have been only a few reports on CK18-F levels with regard to NAFLD in Asian countries [21, 34]. With respect to the development of NASH, the difference in genetic and nongenetic backgrounds of general populations between Asian and Western countries have been suggested [2]; therefore, further studies on the significance of CK18-F levels for NAFLD are required.

In the present study, similar to previous studies, serum CK18-F levels were closely correlated with individual disease components (steatosis, inflammation, and ballooning) and with the overall NAS. Among the individual disease components, ballooning had the strongest correlation with serum CK18-F level. Brunt et al. reported that ballooning is the most significant histological feature in determining a diagnosis of NASH [24]. Since there was a high correlation between the serum CK18-F level and ballooning, CK18-F may be useful for differentiating between NAFL and NASH. However, similar to the previous report [21], our findings indicated that the serum CK18-F level did not associate with advanced fibrosis in NASH.

We tried to determine the serum CK18-F cut-off value for diagnosis of definite NASH compared to NAFL since a value did not previously exist [35]. The optimal cut-off points of serum CK18-F for NAFL and definite NASH were 230 and 270 U/L, respectively. Serum CK18-F (< 230 U/L) performed well as a screening test for NAFL given its high sensitivity (0.89) and high NPV (0.97), while it had low specificity (0.65) and low PPV (0.34). The serum CK18-F (> 270 U/L) did not perform well as a screening test for definite NASH given its low sensitivity (0.64) and NPV (0.67), but did have a relatively higher specificity (0.76) and PPV (0.72). Accuracies of diagnosing both NAFL and definite NASH were comparative.

Although previous reports showed a limited value of using serum CK18-F levels as a biomarker for NASH and fibrosis in patients with NAFLD [36], our findings suggest that serum CK18-F level may be useful for differentiating between NAFL and NASH.

In conclusion, this study established the use of serum CK18-F as a noninvasive biomarker for differentiating between NAFL and NASH. By combining the serum CK18-F level with other noninvasive markers, higher-precision prognosis prediction for NAFLD may be attained.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Yuta Aida, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Jikei University School of Medicine, Katsushika Medical Center, 6-41-2 Aoto, Katsushika-ku, Tokyo 125-8506, Japan. Tel: +81 3 3603 2111; Fax: +81 3 3838 9944; E-mail: aidajik@jikei.ac.jp

## References

[1] Adams LA and Lindor KD. Nonalcoholic fatty liver disease. Ann Epidemiol 2007; 17: 863-869.

[2] Ono M and Saibara T. Clinical features of non-alcoholic steatohepatitis in Japan: Evidence from the literature. J Gastroenterol 2006; 41: 725-732.

[3] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC and McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999; 116: 1413-1419.

[4] Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, Takasaki K and Ludwig J. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002; 37: 154-160.

[5] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M and Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55: 2005-2023.

[6] Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001; 121: 710-723.

[7] Nascimbeni F, Pais R, Bellentani S, Day CP, Ratziu V, Loria P and Lonardo A. From NAFLD in clinical practice to answers from guidelines. J Hepatol 2013; 59: 859-871.

[8] Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM and Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004; 40: 1387-1395.

[9] Iijima H, Moriyasu F, Tsuchiya K, Suzuki S, Yoshida M, Shimizu M, Sasaki S, Nishiguchi S and Maeyama S. Decrease in accumulation of ultrasound contrast microbubbles in non-alcoholic steatohepatitis. Hepatol Res 2007; 37: 722-730.

[10] Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S and Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with non-

alcoholic fatty liver disease (NAFLD). Dig Liver Dis 2008; 40: 371-378.
[11] Kumar R, Rastogi A, Sharma MK, Bhatia V, Tyagi P, Sharma P, Garg H, Chandan Kumar KN, Bihari C and Sarin SK. Liver stiffness measurements in patients with different stages of nonalcoholic fatty liver disease: diagnostic performance and clinicopathological correlation. Dig Dis Sci 2013; 58: 265-274.
[12] Forns X, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM and Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36: 986-992.
[13] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS and Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518-526.
[14] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D and Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325.
[15] Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A and Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006; 6: 33.
[16] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM and Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007; 45: 846-854.
[17] Harrison SA, Oliver D, Arnold HL, Gogia S and Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008; 57: 1441-1447.
[18] Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M and Yki-Järvinen H. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009; 137: 865-872.
[19] Bantel H, Lügering A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, Manns MP and Schulze-Osthoff K. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. Hepatology 2004; 40: 1078-1087.
[20] Papatheodoridis GV, Hadziyannis E, Tsochatzis E, Georgiou A, Kafiri G, Tiniakos DG, Margariti K, Manolakopoulos S, Manesis EK and Archimandritis AJ. Serum apoptotic caspase activity in chronic hepatitis C and nonalcoholic Fatty liver disease. J Clin Gastroenterol 2010; 44: e87-95.
[21] Tsutsui M, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E and Sano K. Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. J Clin Gastroenterol 2010; 44: 440-447.
[22] Kojima S, Watanabe N, Numata M, Ogawa T and Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. J Gastroenterol 2003; 38: 954-961.
[23] WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004 Jan; 363: 157-163.
[24] Brunt EM, Kleiner DE, Wilson LA, Belt P and Neuschwander-Tetri BA; NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011; 53: 810-820.
[25] Tanaka N, Tanaka E, Sheena Y, Komatsu M, Okiyama W, Misawa N, Muto H, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Horiuchi A and Kiyosawa K. Useful parameters for distinguishing nonalcoholic steatohepatitis with mild steatosis from cryptogenic chronic hepatitis in the Japanese population. Liver Int 2006; 26: 956-963.
[26] Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
[27] Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T and Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000; 23: 57-63.
[28] Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ and Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 2006; 44: 27-33.
[29] Yilmaz Y, Dolar E, Ulukaya E, Akgoz S, Keskin M, Kiyici M, Aker S, Yilmaztepe A, Gurel S,

Gulten M and Nak SG. Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. World J Gastroenterol 2007; 13: 837-844.

[30] Malik R, Chang M, Bhaskar K, Nasser I, Curry M, Schuppan D, Byrnes V and Afdhal N. The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. J Gastroenterol Hepatol 2009; 24: 564-568.

[31] Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L and Kroemer G. Cell death modalities: classification and pathophysiological implications. Cell Death Differ 2007; 14: 1237-1243.

[32] Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD and Gores GJ. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003; 125: 437-443.

[33] Francque SM, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, Michielsen P and Van Gaal L. Noninvasive assessment of nonalcoholic fatty liver disease in obese or overweight patients. Clin Gastroenterol Hepatol 2012; 10: 1162-1168; quiz e87.

[34] Shen J, Chan HL, Wong GL, Chan AW, Choi PC, Chan HY, Chim AM, Yeung DK, Yu J, Chu WC and Wong VW. Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers. Aliment Pharmacol Ther 2012; 36: 1057-1066.

[35] Yoneda M. Is the measurement of serum cytokeratin-18 useful for diagnosis or screening non-alcoholic steatohepatitis? Hepatol Res 2014; 44: 827-828.

[36] Cusi K, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M and Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol 2014; 60: 167-174.